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ORIGINAL PAPER

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Aronia melanocarpa phenolics and their antioxidant activity

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Abstract The purpose of the present study was to evaluate small and high molecular phenolics (tannins) and antioxidant activity of *Aronia melanocarpa* berries, juice and pomace in order to find new potential sources of natural antioxidants. The fruits of *Aronia melanocarpa* Elliot were collected in the middle of October at a plantation near Wroclaw, Poland. The pomace has a much higher content of phenolics in comparision to juice and fruits. Results showed that polymeric proanthocyanins, predominantly of (−)epicatechin, are the major class of polyphenolic compounds in chokeberry, represent 66% of fruits polyphenols. The average concentration ranged from 1578.79 mg/100 g of DW for chokeberry juice up to 8191.58 mg/100 g in pomace. The concentration of phenolic acids (chlorogenic and neochlorogenic acids) in juice was higher than in pomace. Anthocyanins in *Aronia melanocarpa* are second phenolic compound group and represent about 25% of total polyphenols, mixture of four different cyanidin glycosides: 3-galactoside, 3-glucoside, 3-arabinoside and 3-xyloside. The higher antioxidant activity expressed as TEAC was measured in pomace >fruit >juice.

Keywords *Aronia melanocarpa* · Phenolic acids · Tannin · Anthocyanin glycosides · Antioxidant

Introduction

The potential sources of antioxidant phenolics have been searched in several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs, and crude plant drugs [1]. Berries are known as plant material very rich in phenolics. Berries and fruits contain a wide range of flavonoids and phenolic acids that show antioxidant activity. Main flavonoid subgroups in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries and fruits are hydroxylated derivates of benzoic acid and cinnamic acid [2].

Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease. Consumption of controlled diets high in fruits and berries significantly increased the antioxidant capacity of plasma [3]. Moreover, epidemiological studies have found that there is a significant negative relation between the intake of fruits and vegetables and heart disease mortality [4, 5].

The importance of the antioxidant constituents of berries in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward fruits with specific health effects [6]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential [7].

Studies on antioxidative activities of fruit extracts have been focused mainly on grapes, which have been reported to inhibit oxidation of human low density lipoprotein (LDL) at a level comparable to wine [8]. Fresh strawberry extract was reported to have 15 times higher total antioxidant capacity than trolox in an artificial peroxyl radical model system [9]. Extracts of blackberries, black and red currants, blueberries, and black and red raspberries possessed a remarkably high scavenging activity toward chemically generated superoxide radicals [10]. Hydroxycinnamic acids typically present in fruits have been shown to inhibit LDL oxidation in vitro [11]. Also phenolic extracts of berries (blackberries, red raspberries, sweet cherries, blueberries and strawberries) were shown to inhibit human low-density lipoprotein (LDL) and liposome oxidation [12].

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The high antioxidant activity was also reported for a few known berries, such as chokeberry *Aronia melanocarpa* [13–15] that correlate with high phenolic content. The chokeberry had significantly higher anthocyanin, phenolic content and antioxidant activity than the blueberry, cranberry and lingonberry crops [13]. It was shown that red pigment fraction of black chokeberry has a potent antioxidative activity in both in vitro and in vivo systems and played a significant role in antiulcerative activity on acute gastric hemorrhagic lesions in rats [16]. The anthocyanin level of these berries has been reported to be as high as 1% on a dry weight basis [17] and total phenolic content more than 20 mg/g (gallic acid equivalents) [14]. Four anthocyanins: 3-O-galactoside, 3-O-glucoside, 3-O-arabinoside and 3-O-xyloside of cyanidin [17, 18], two phenolic acids: chlorogenic and neochlorogenic [19] and, recently, five quercetin derivatives [20] were determined in chokeberry fruits. Their are astringent in taste, that indicate on tannins content in these fruits. The concentration of chokeberry condensed tannins was higher than in the other berries [21]. They contain mainly (−)epicatechins as subunits, that are named procyanidins [22].

Proanthocyanidins are of great interest in nutrition and medicine because of their potent antioxidant capacity and possible protective effects on human health [23]. It has recently been hypothesized that the free radical scavenging properties of procyanidins may reduce the risk of cardiovascular diseases [25, 26], cancer [24], blood clotting [27] and certain types of trimeric PAs may protect against urinary tract infections [28].

Aronia melanocarpa (Michx) Elliott belong to the Rosaceae family, native to eastern North America, is a 0.5±3 m high shrub. The black berries are borne in clusters and ripen in early September in Poland. They are used for production of jam, juice, wine and anthocyanin colorant.

The purpose of the present study was to evaluate small and high molecular phenolics (tannins) and antioxidant activity of *Aronia melanocarpa* berries, juice and pomace in order to find new potential sources of natural antioxidants.

The ability of small phenolics, including flavonoids and phenolic acids, to act as antioxidants has been extensively investigated [7] but the high molecular weight phenolics known as tannins have been neglected.

Materials and methods

Plant material

Fruits of *Aronia melanocarpa* Elliot were collected in the middle of October at a plantation near Wroclaw, Poland, and immediately stored at −20 °C.

Sample preparation

Chokeberries were thawed overnight at room temperature. The fruit was crushed and heated at 80 °C during 5 min using a Thermomix (Vorwerk, Germany). The pulp, after cooling, was depectinized at 50 °C for 1 h by adding 3 ml pectinase (Rapidase Super BE; Gist-Brocades Laboratories, Charlotte, N.C., U.S.A.) per kg. Pressing of the pulp was done at a final pressure of 300 kPa for 10 min in a laboratory hydraulic press (Type Zoodiak). The juice was pasteurized at 85–90 °C for 10 min in 0.2 l jars water bath heater and next cooled to 20 °C. All processing procedures were replicated.

The chokeberry juice was diluted 10 times in redistilled water and filtered through 0.45 μm filters (Millipore, Bedford, MA) for HPLC analysis. Frozen berries and pomace were lyophilized. After grinding, 1 g samples were extracted with methanol acidified with 0.1% HCl. The extraction was performed by sonification for 20 min with occasionally shaking in ultrasonic bath. This has proved to be adequate for complete extraction. Next, the slurry was centrifuged at 19000×*g* for 10 min and the supernatant was used for HPLC analysis. The supernatant was recovered and filtered through a 0.45 μm cellulose syringe filter before analysis.

HPLC analyses

The HPLC analysis of (−)epicatechin, hydroxycinnamates, anthocyanin glycosides and flavonol glycosides were carried out on a HPLC apparatus consisting of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The separation was performed on a Synergi Fusion RP-80A 150×4.6 mm (4 μm) Phenomenex (Torrance, CA USA) column, Column oven temperature was set to 30 °C. The mobile phase was composed of solvent A (2.5% acetic acid) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 36 min 25% B, followed by washing and reconditioning the column. The flow rate was 1 ml min^{-1}, and the runs were monitored at the following wavelenghts: (−)epicatechin at 280 nm, chlorogenic and neochlorogenic acid at 320 nm, flavonol glycosides at 360 nm, and anthocyanin glycosides at 520 nm. The Photo Diode Array (PAD) spectra were measured over the wavelength range 240–600 nm in steps of 2 nm. Retention times and spectra were compared to those of pure standards within 200–600 nm.

In addition, an enzymatic hydrolysis of flavonol and anthocyanin glycosides was performed for identification. The lyophilized powdered berries were mixed with the citrate buffer solution at pH 5. Afterward, specific enzymes were added: β-glucosidase, β-xylosidase, β-galactosidase, and β-hesperidinase (Sigma, Steinheim, Germany). The disappearance of single peaks in the chromatogram and formation of the corresponding aglycon was observed using HPLC after 1-h incubation at 38 °C with a specific enzyme.

Quantification

The amounts of anthocyanins, chlorogenic acids and flavonols in the samples were analyzed by HPLC. Standard

curves made from cyanidin 3-galactoside and chlorogenic acid supplied by Polyphenols Laboratories were used for the analysis of anthocyanins and chlorogenic acids, respectively, while flavonols were assayed from that of standard rutin (Sigma-Aldrich).

Proanthocyanidins analysis

The direct thiolysis on freeze-dried juice, berries and pomace was performed [29]. Powders are precisely weighed (30–50 mg) in 1.5 ml Eppendorf vials, acidic methanol (3.3% (v/v), 400 μl) and toluene α-thiol (5% in methanol, 800 μl) are added. The vials are closed and incubated at 40 °C for 30 min with agitation on vortex every 10 min. Then, the vial are cooled in ice water and centrifuged immediately at 4 °C refrigerated centrifuge during 10 min. Samples are stored at 4 °C until RP-HPLC analysis. All incubations were performed in triplicate.

The thiolysis products separated on Merck Purospher RP 18 end-capped column 250×4 mm, 5 μm (Merck, Darmstadt, Germany). The HPLC apparatus is a Waters (Milford, MA) systems (DAD and Scanning Fluorescence detectors). The solvent system is a gradient of solvent A (aqueous acetic acid, 2.5%. v/v) and solvent B (acetonitrile) and the following gradient is applied: initial 3% B, 0–5 min, 9% B linear; 5–15 min, 16%B linear; and 15–45 min, 50%B linear, followed by washing and reconditioning the column. The flow rate 1 ml min^{-1}, and oven temperature 30 °C were used. Compounds, for which reference standards were available, were identified on chromatograms according to their retention times and UV-visible spectra. The fluorescence detection was recorded at excitation wavelength 278 nm and emission wavelength 360 nm. The calibration curves (based on peak area at 280 nm) were established using flavan-3-ol and benzylthioether standards. The average degree of polymerization was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts + terminal units) to (−)epicatechin and (+)-catechin corresponding to terminal units.

Scavenging effect on DPPH and ABTS radicals

The DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and potassium sulfate (di-potassium peroxdisulfate) were obtained from Sigma-Aldrich Co., U.S.A.

The effect of juice, berries, and pomace extracts on DPPH radical was determined according to the method of Yen and Chen [30]. A 1 g of freeze-dried sample was homogenized in 20 ml of methanol. The slurry was filtered and filtrate was diluted in methanol. A 1 ml aliquot of diluted extract was added to 3 ml of absolute methanol and 1 ml of DPPH (0.012 g DPPH/100 ml). The mixture was shaken and left at room temperature for 10 min; the absorbance was measured spectrophotometrically at 517 nm.

The effect of juice and freeze-dried berries and pomace extracts on ABTS radical was measured according to Re et al. [31]. ABTS stock solution (7 mM concentration) and 2.45 mM potassium persulfate were left at room temperature for 16 h to produce ABTS radical cation ($ABTS^{\cdot+}$). A 1 g of sample was homogenized in 50 ml of absolute methanol and filtered. $ABTS^{\cdot+}$ solution was diluted with distilled water to an absorbance of 0.700 (±0.02) at 734 nm. A 1 ml of $ABTS^{\cdot+}$ solution was added to 10 μl of sample extract; the absorbance was monitored at 734 nm after 6 min. A concentration-response curve for the absorbance at 734 nm after 6 min for $ABTS^{\cdot+}$ as a function of different Trolox concentrations was prepared. The decrease in absorption at 734 nm 6 min after addition of the compound was used for calculating the TEAC (Trolox equivalent antioxidant capacity).

Results and discussions

The results of qualitative and quantitative phenolic composition in black chokeberries fruits, juice and pomace determined by HPLC method are presented in Table 1. The pomace has a much higher content of phenolics than juice and fruits.

Aronia melanocarapa is known as a very rich source of anthocyanins and phenolic acids. Our results showed that polymeric proanthocyanins are the major class of polyphenolic compounds in chokeberry (Table 1 and Fig. 1) and represent 66% of fruits polyphenols. The average concentration ranged between 1578.79 mg/100 g of DW for chokeberry juice up to 8191.58 mg/100 g in pomace. Polymeric flavan-3-ols of *Aronia melanocarpa* are composed predominantly of (−)epicatechin as constitutive units of

Table 1 Phenolic compounds distribution (mg/100 g dried weight) and antioxidant activity of *Aronia melanocarpa* Elliot

Compounds	Fruits	Pomace	Juice
Chlorogenic acid	301.85	204.35	415.86
Neochlorogenic acid	290.81	169.20	393.10
(−)epicatechin	15.04	11.41	12.71
Polymeric procyanidins	5181.60	8191.58	1578.79
Degree polymerization DP	23	34	23
Quercetin 3-rutinoside	15.10	13.55	27.53
Quercetin 3-galactoside	36.98	47.44	49.76
Quercetin 3-glucoside	21.64	26.50	31.24
Quercetin derivatives unidentified	27.43	82.40	46.93
Cyanidin 3-galactoside	1282.41	1119.70	787.00
Cyanidin 3-glucoside	42.14	79.44	28.15
Cyanidin 3-arabinoside	581.50	532.64	324.37
Cyanidin 3-xyloside	52.71	105.06	33.63
Total phenolics	7849.21	10583.27	3729.07
Antioxidant activity (μM Trolox/100 g dried weight)			
DPPH radicals	279.38	301.89	127.45
ABTS radicals	439.49	779.58	314.05

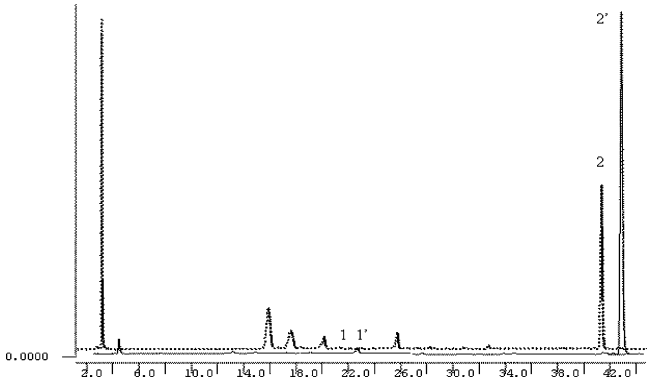

Fig. 1 Chromatograms HPLC (detectors: fluorescence—continuous line; absorbance UV 280 nm—dotted line) of *Aronia melanocarpa* fruit thiolytic degradation products 1. 1′-(−)epicatechin; 2. 2′(−)epicatechin-α -benzyl thioether

procyanidins. The chain extension units and chain terminating units in procyanidins were predominant as (−)epicatechin (Fig. 1).

In chokeberry fruits, the anthocyanins are the second phenolic compound group and represent about 25% of total polyphenols. Anthocyanins in *Aronia melanocarpa* are a mixture of four different cyanidin glycosides: 3-galactoside, 3-glucoside, 3-arabinoside and 3-xyloside of which cyanidin 3-galactoside is the main one. These results are in agreement with the previous studies [18].

Chlorogenic and neochlorogenic acids represent 7.5% of aronia fruit polyplenols. The main compound was 5′-caffeoyl quinic acid (chlorogenic acid). The results confirmed our earlier reports [19]. The concentration of phenolic acids in juice was higher than in pomace which indicated its good solubility in water.

The content of flavonols and (−)epicatechin in chokeberry fruits was low compared to the content of anthocyanins, chlorogenic acids and procyanidins. Flavonols in chokeberry are a mixture of five different quercetin glycosides recently identified [20]. We identified only three of them the derivative 3-rutinoside, 3-galactoside and 3-glucoside. Flavonols represent only 1.3% of total chokeberry fruit phenolics.

The results presented in Table 1 indicated high antioxidant activity of chokeberry fruits with large variation ranging from 127.45 (juice) to 301.89 (pomace) for DPPH radicals equivalents per μM Trolox/100 g dried weight and from 314.05 (juice) to 779.58 (pomace) μM Trolox/100 g dried weight for ABTS radical. These plants have a very high antioxidant activity, comparable to traditional Chinese medicinal plants associated with anticancer activity [32]. Kahkonen et al. [33], after examination of the phenolic contents and antioxidative activity in 92 plant extracts, reported that among edible plant materials, remarkable high antioxidant activity and high total phenolic content (GAE. 20 mg/g) were found in berries, especially aronia and crowberry.

Our results show that *Aronia melanocarpa* berries are very rich in o-diphenolics as caffeic acids, (−)epicatechin, cyanidin and quercetin derivatives. These compounds are the most active as antioxidants, because the o-dihydroxy structure in the B ring confers higher stability to the radical form and participates in electron delocalization [7]. The high molecular weight of chokeberry procyanidins containing many aromatic rings and hydroxyl groups are also important for free radical scavenging. Hagerman et al. [34] reported, that tannins may be much more potent antioxidants than simple monomeric phenolics.

Our result showed that *Aronia melanocarpa* berries and their products, such as juice and pomace contained a high level of polyphenols with high antioxidant activity, which could be commercially exploited. This plant might be a potential source of potent natural antioxidants.

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References

1. Ramarathnam N, Ochi H, Takeuchi M (1997) Antioxidant defense system in vegetable extracts. In natural antioxidants: chemistry. health effects. and applications. Shahidi F. AOCS Press: Champaign. IL, pp 76–87
2. Macheix JJ, Fleuriet A, Billot J (1990) Fruit Phenolics. CRC Press: Boca Raton, FL
3. Cao G, Booth SL, Sadowski JA, Prior RL (1998) Am J Clin Nutr 68:1081–1087
4. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D (1993) Lancet 342:1007–1011
5. Knekt P, Jarvinen R, Reunanen A, Maatela J (1996) Br Med J 312:478–481
6. Loliger J (1991) The use of antioxidants in food. In: Aruoma OI, Halliwell B (eds) Free Radicals and Food Additives. Taylor and Francis, London, pp 129–150
7. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB (1995) Free Radical Res 22:375–383
8. Meyer AS, Yi OS, Pearson DA, Waterhouse AI, Frankel EN (1997) J Agric Food Chem 45:1638–1643
9. Wang H, Cao G, Prior RL (1997) J Agric Food Chem 45:304–309
10. Constantino L, Albasino A, Rastelli G, Benvenuti S (1992) Planta Med 58:342–344
11. Meyer AS, Donovan JL, Pearson DA, Waterhouse AI, Frankel EN (1998) J Agric Food Chem 46:1783–1787
12. Heinonen IM, Meyer AS, Frankel EN (1998) J Agric Food Chem 46:4107–4112
13. Zheng W, Wang S (2003) J Agric Food Chem 2003 51:502–509
14. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999) J Agric Food Chem 47:3954–3962
15. Gasiorowski K, Szyba K, Brokos B, Kołaczyńska B, Jankowiak-Wlodarczyk M, Oszmiański J (1997) Cancer Letters 119:37–46
16. Matsumoto M, Hara H, Chiji H, Kasai T (2004) J Agric Food Chem 52:2226–2229
17. Strigl AW, Leitner E, Pfannhauser W (1995) Zeit Lebens Unters Forsch 91:177–180
18. Oszmiański J, Sapis JC (1988) J Food Sci 53(4):1241–1242
19. Oszmiański J, Sapis JC (1989) Zesz Nauk AR Wrocław Techn Żywn V 184:75–87
20. Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Giske NH (2005) J Food Compos Anal 18:61–68
21. Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Gebhardt S, Prior RL (2004) J Nutr 134(3):613–617
22. Oszmiański J, Kucharska A (1995) Zesz Nauk AR Wrocław Techn Żywn VIII 273:75–87

23. Santos-Buelga C, Scalbert A (2000) J Sci Food and Agric 80:1094–1117
24. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA (2000) Toxicology 148(2–3):187–197
25. Reed J (2002) Crit Rev Food Sci Nutr 42(Suppl) 301–316
26. Steinberg F, Bearden M, Keen C (2003) J Am Diet Assoc 103:215–223
27. Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Moriarty H, Pike MJ, Turner AH, Mann NJ, Sinclair AJ (2003) Am J Clin Nutr 77:1466–1473
28. Foo IY, Lu Y, Howell AB, Vorsa N (2000) J Nat Prod 63:1225–1228
29. Guyot S, Marnet N, Sanoner P, Drilleau JF (2001) Methods in Enzymology 335:57–70
30. Yen GC, Chen HY (1995) J Agric Food Chem 43:27–32
31. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Free Radical Biology&Medicine 26(9/10):1231–1237
32. Cai Y, Luo Q, Sun M, Corke H (2004) Life Sci 74:2157–2184
33. Hakkinen SH, Karenlampi SO, Heinonen M, Mykkanen HM, Torronen AR (1999) J Agric Food Chem 47:2274–2279
34. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL (1998) J Agric Food Chem, 46:1887–1892